Species Specificity and Augmentation of Responses to Class II Major Histocompatibility Complex Molecules in Human CD4 Transgenic Mice

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Summary

Murine T cell responses to human class II major histocompatibility complex (MHC) molecules were shown to be a minimum of 20–70-fold lower than responses to allogeneic molecules. Transgenic mice expressing slightly below normal (75–95%) or very high (250–380%) cell surface levels of human CD4 were utilized to determine whether this was due to a species-specific interaction between murine CD4 and class II molecules. Human CD4 was shown to function in signal transduction events in murine T cells based on the ability of anti-human CD4 antibody to synergize with suboptimal doses of anti-murine CD3 antibody in stimulating T cell proliferation. In mice expressing lower levels of human CD4, T cell responses to human class II molecules were enhanced up to threefold, whereas allogeneic responses were unaltered. In mice expressing high levels of human CD4, responses to human class II molecules were enhanced at least 10-fold, whereas allogeneic responses were between one and three times the level of normal responses. The relatively greater enhancement of the response to human class II molecules in both lines argues for a preferential interaction between human CD4 and human class II molecules. In mice expressing lower levels of human CD4, responses to human class II molecules were blocked by antibodies to CD4 of either species, indicating participation by both molecules. In mice expressing high levels of human CD4, responses to both human and murine class II molecules were almost completely blocked with anti-human CD4 antibody, whereas anti-murine CD4 antibody had no effect. However, anti-murine CD4 continued to synergize with anti-CD3 in stimulating T cell proliferation in these mice. Thus, overexpression of human CD4 selectively impaired the ability of murine CD4 to assist in the process of antigen recognition. The ability of human CD4 to support a strong allogeneic response under these conditions indicates that this molecule can interact with murine class II molecules to a significant extent. Despite the fact that human CD4 appeared to be the only functional coreceptor in these mice, responses to human class II molecules were still much lower than those to murine class II alloantigens. This indicates that species-specific interactions between class II molecules and CD4 expressed on peripheral T cells are not sufficient to account for the low xenogeneic response and that intrinsic differences in T cell receptor structures or the need for species specificity in the interaction between CD4 and class II molecules during positive selection are also important.

The response of T cells of one species to cells of another has been shown to be restricted entirely to recognition of the MHC products on the stimulator cells (1–3). Similar to allogeneic and MHC-restricted responses, proliferative responses to xenogeneic class II MHC are generally mediated by $CD4^+$ lymphocytes (4, 5), whereas CTL responses to xenogeneic class I molecules are mediated by $CD8^+$ cells (5, 6). Such responses are directed against "alloantigenic" determinants rather than to species-specific epitopes as demonstrated by the predominant response of xenogeneic T cells to polymorphic determinants of the MHC antigens of another species (1, 2, 5, 7–11). However, murine T cell responses to human class I (12, 13) and class II molecules (2–4, 14) are one to two orders of magnitude lower than responses against allogeneic MHC molecules.

Thymic MHC molecules have been shown to participate in the positive selection of MHC-restricted (15–17) and alloreactive (18) T cells. Thus, one possible explanation for the

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low murine T cell response to human MHC molecules is the absence in the murine thymus of a restriction element capable of selecting appropriate T cell receptors. However, the frequencies of CTL precursors responding to human class I molecules in human class I transgenic mice were comparable to those of normal mice (19, 20). Similarly, expression of the human DQ3.2 molecule in transgenic mice did not enhance their response to human class II molecules (14). These results indicate that the expression of human MHC molecules in the thymus does not enhance the selection of human MHC-specific murine T cells.

Another possible explanation is based on the low sequence homology between murine and human homologues of CD4 (21), which might result in low avidity interactions between a T cell and a stimulator cell of a different species. Thus, the low frequency of murine T cells responding to human MHC molecules could be due to a poor interaction between the murine CD4 and CD8 coreceptor molecules and human class II and class I molecules, respectively. This hypothesis is supported by studies showing enhanced murine CTL responses to human class I molecules when the $\alpha 3$ domain was replaced with one of murine origin (20, 22, 23). However, in other studies, the species of origin of the α 3 domain of class I molecules did not influence the frequency of CTL precursors or the efficiency of T cell recognition (13, 24, 25). In this paper, we have investigated whether the low murine T cell response to human class II molecules is due to species specificity in the binding of murine CD4. In contrast to previous approaches, we have utilized transgenic mice expressing human CD4 in order to determine the influence of this molecule on responses to both human and murine class II molecules.

Materials and Methods

Transgenic Mice. Constructs containing the human CD4 gene were microinjected into fertilized eggs from (C57BL/6 × CBA)F_2 mice, and positive founder mice from each transgenic line were backcrossed with C57BL/6 (H-2^b) mice four to five times. Line 37 contains approximately three copies of the human CD4 gene expressed under the control of the proximal $p56^{lck}$ promoter, whereas line 996 contains approximately 20 copies expressed under the control of the CD3 δ enhancer and promoter (Grass, D. S., A. Garvin, D. Littman, N. Lee, and E. Lacy, manuscript submitted for publication). Northern analysis has indicated that human CD4 gene expression is primarily restricted to T cells. HLA-DQ3.2 transgenic mice were generated as previously described using fertilized eggs from C57BL/10 (H-2^b) mice (14). The DQ3.2 molecule is expressed on splenic B cells at slightly higher levels than those found on human PBL (data not shown).

Cell Lines. A20.2J is a BALB/c (H-2^d)-derived B cell line. The human B lymphoblastoid cell lines C1R (26) (HLA-A negative, -B negative, -Cw4, -DR8, -DPw4, DQ3) and LCL 721.221 (27) (class I negative, -DR1, -DQ1, -DP2) were immunoselected for loss of expression of class I MHC molecules.

Monoclonal Antibodies. Leu3a (anti-human CD4) (gift of L. Lanier; Becton Dickinson and Co., Mountain View, CA), GK1.5 (anti-murine CD4), L243 (anti-HLA-DR monomorphic), and 34-2-12 (anti-H-$2D^d$) were purified and used at a final concentration of 20 μg/ml). 145-2-C11 (anti-murine CD3), 2.43 (anti-murine CD8) and MKD6 (anti-I-A^d) were used as culture supernatants, whereas IV-D-12 (anti-HLA-DQ monomorphic) was used as a 1:100 dilution of ascites fluid.

Cell-Surface Expression of CD4. Murine spleen cells or human PBL were incubated with Leu3a or GK1.5 antibody in RPMI 1640 containing 5% FCS and 0.02% sodium azide for 30 min at 4°C. Cells were washed twice and incubated with a 1:50 dilution of fluorescein-conjugated goat anti-mouse IgG antibody, Fc specific (Cappel Laboratories, Cochranville, PA). After two washes, the cells were fixed with 0.5% paraformaldehyde in PBS. Fluorescence intensity was determined using a FACScan®.

Mixed Lymphocyte Response. Spleen cells (5×10^5) from transgenic mice or littermate controls were cultured with the indicated numbers of irradiated B cell lines or spleen cells in RPMI 1640 containing 10% FCS and 5×10^{-5} M 2-ME in 96-well plates. Cells were cultured in the presence or absence of the indicated antibodies. Cultures were maintained for 4 d at 37°C and pulsed with 1 μCi of [^{3}H]thymidine for the last 16–18 h. Cells were harvested onto glass fiber filters using a PhD cell harvester and counted. Results represent the mean of triplicate cultures (±SD) after subtraction of background responses of stimulator cells in the absence of responders.

Antibody-mediated T Cell Proliferation. The ability of antibodies to human and murine CD4 to synergize with suboptimal concentrations of anti-CD3 antibodies was determined as previously described (28, 29). Flat-bottomed 96-well plates were coated with 10 μg/ml of sheep anti-mouse IgG with or without 10 μg/ml of rabbit anti-rat IgG antibody at 4°C. After washing the plates to remove unbound antibody, 0.2 μg/ml of Leu 3a (binds to anti-mouse IgG) or a 1:100 dilution of GK1.5 culture supernatant (binds to anti-rat IgG) was added simultaneously with the indicated dilutions of 145-2-C11 antibody (binds to anti-mouse IgG). As negative controls either X63 culture supernatant that contains murine IgG or 0.2 μg/ml of rat IgG were added together with various dilutions of 145-2-C11. After 1 h at 37°C, unbound antibody was removed, and 3×10^4 purified T cells from either transgenic mice or littermate controls were added to each well. T cells were purified by adherence to plastic for 1 h, nylon wool column passage, and treatment with anti-I-A^b antibody 25-5-16 plus rabbit complement to deplete remaining B cells. T cell purity was determined to be >92% by staining with antibody to CD3. Cells were cultured with 100 U/ml of rIL-2 for 4 d, pulsed for the last 16 h with 1 μCi of [^{3}H]thymidine, and harvested onto glass fiber filters. Results represent the mean of triplicate cultures (±SD).

Results

Expression of Human CD4 Molecules in Transgenic Mice. The level of cell surface expression of human CD4 in transgenic mice from lines 37 and 996 was compared to its level of expression on human PBL by flow cytometry. Analysis of four mice from each of the two transgenic lines in different experiments revealed that line 37 expresses human CD4 at 75–95% of the level found on human PBL from three different donors (Fig. 1 *A*). The level of human CD4 expression in purified T cells from line 996 was three- to fourfold the level found on T cells from line 37 (Fig. 1 *B*) and 2.5–3.8-fold the level found on human PBL (Grass, D. S., A. Garvin, D. Littman, N. Lee, and E. Lacy, manuscript submitted for publication). By comparison with normal littermates, the percentage of cells expressing murine CD4 and CD8 was not altered in either of these two lines (Grass, D. S., A. Garvin,

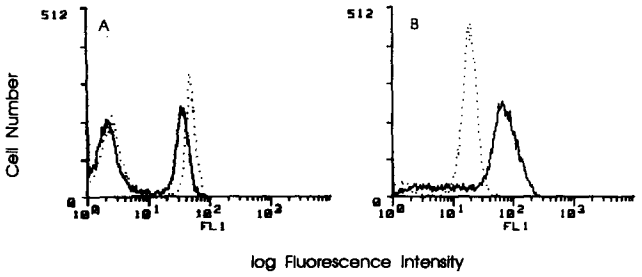

Figure 1. Level of expression of human and murine CD4 in transgenic mice. (*A*) Human CD4 expression on spleen cells from line 37 transgenic mice (*solid line*) and human PBL (*dotted line*). (*B*) Human CD4 expression on purified T cells from line 996 (*solid line*) and from line 37 (*dotted line*). Forward angle light scatter profiles for all cells were comparable, indicating that the cells are of similar size. Experimental details are given in Materials and Methods.

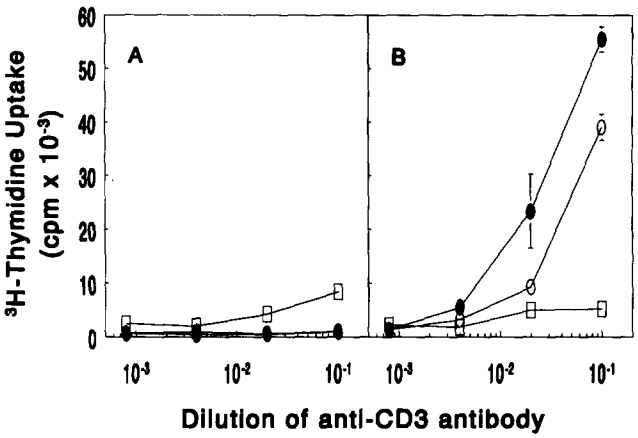

Figure 2. Human CD4 synergizes with murine CD3 to induce proliferation. Purified splenic T cells from littermate control (*open squares*), line 37 (*open circles*), and line 996 (*closed circles*) were cultured in plates coated with serial dilutions of anti-CD3 together with either a negative control antibody (*A*) or anti-human CD4 (*B*) in the presence of 100 U/ml of recombinant IL-2 for 4 d. Bars, SD. Further experimental details are given in Materials and Methods.

D. Littman, N. Lee, and E. Lacy, manuscript submitted for publication). However, in both lines the level of expression of murine CD4 was reduced by 50%.

Participation of Human CD4 in Signal Transduction Events. It has been previously shown that T cells cultured in the presence of suboptimal concentrations of anti-CD3 or anti-TCR antibodies can be induced to proliferate by the simultaneous addition of antibodies to CD4 or CD8 (28, 29), indicating that crosslinking CD4 or CD8 with the TCR triggers signal transduction events and results in T cell activation. This experimental assay was utilized to determine whether human CD4 expressed in transgenic mice interacts with murine components involved in signal transduction and proliferation. Simultaneous addition of anti-human CD4 antibody with concentrations of anti-CD3 antibody that do not stimulate T cell proliferation alone (Fig. 2 *A*) resulted in strong proliferative responses in T cells from both transgenic lines (Fig. 2 *B*). Comparable levels of T cell proliferation were obtained with 2.2-fold less anti-CD3 antibody in line 996 as compared to line 37, whereas the former expressed three- to fourfold as much CD4. T cells from littermate control animals did not proliferate in response to simultaneous additions of anti-human CD4 and anti-CD3 antibodies (Fig. 2 *B*). These results indicate that human CD4 expressed in transgenic mice interacts with murine components required for T cell activation.

T Cell Responses to Class II Molecules in Human CD4 Transgenic Mice. It has been previously demonstrated that murine T cell responses to human class II molecules are much lower than responses to allogeneic class II molecules (4, 14, 30). In normal C57BL6 (H-2^b) mice, T cell responses to human class II-positive B cell lines C1R and 721.221 were still considerably lower than responses to the allogeneic murine B cell line A20.2J even when 35 times more human stimulator cells were used (Fig. 3, compare *open triangles* in *A–C*). In other experiments, significantly lower responses have been observed using up to 70 times more human cells (data not shown). These allogeneic and xenogeneic responses were shown to be primarily directed at class II molecules based on their inhibition by antibodies directed against I-A^d and HLA-DR, respectively (Table 1). Anti-D^d antibody had no effect on the allogeneic response, whereas the cell lines used for xenogeneic stimulation were largely or entirely class I negative. The low response to human class II molecules was not due to a low level of surface expression, since FACS® analysis using several mAbs indicated that 721.221 and C1R expressed similar or higher class II levels than those found

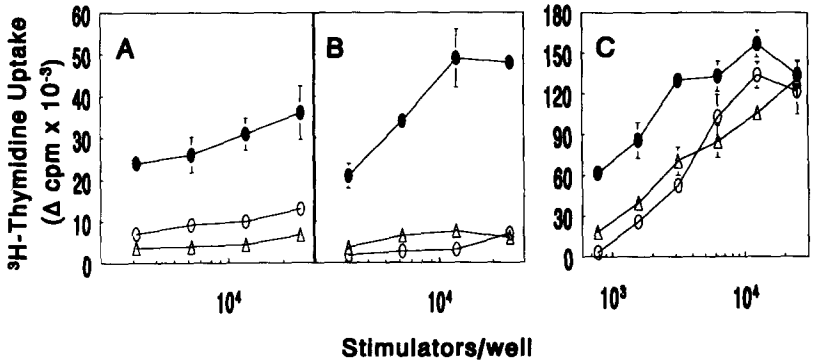

Figure 3. Responses to human and murine class II molecules in normal and CD4 transgenic mice. Spleen cells from line 996 (*closed circles*), line 37 (*open circles*), and littermate control (*open triangles*) were cultured for 4 d with the indicated numbers of irradiated lymphoblastoid cell lines. (*A*) 721.221 (class I negative, -DR1, -DQ1, -DP2). (*B*) C1R (HLA-A negative, -B negative, -CW4, -DR8, -DPw4,DQ3). (*C*) A20.2J (H-2^d). Each data point represents the mean of triplicate cultures after subtraction of the proliferative response of stimulator cells (150–400 cpm for A20.2J and C1R and 400–1500 cpm for 721.221). Proliferative responses in the absence of stimulators ranged from 180 to 300 cpm. Bars, SD.

Table 1. *Proliferative Responses to Human and Murine Cells Are Specific for Class II Molecules*

Stimulator	mAb	Specificity	Responder [^{3}H]thymidine uptake (Δ cpm)					
			B6	% inh*	Line 37	% inh	line 996	% inh
A20 (murine)	None		34,942		102,675		60,380	
	MKD6	I-A^d	12,454	**64**	16,709	**84**	13,594	**77**
	L243	DR	42,834	0	83,904	18	45,876	24
	34-1-2	D^d	34,652	1	ND		63,967	0
721.221 (human)	None		5,087		8,951		27,305	
	L243	DR	2,212	**56**	5,348	**40**	3,452	**87**
	MKD6	I-A^d	3,640	28	ND		20,650	24
	34-1-2	D^d	7,603	0	ND		47,927	0

5 × 10^5 responder spleen cells were stimulated with 6.25 × 10^3 irradiated (5,200 rad) A20 cells, 2.5 × 10^4 irradiated (9,000 rad) 721.221 cells, or 5 × 10^5 irradiated (2,800 rad) spleen cells from DQ3.2 transgenic mice either in the presence or absence of the indicated antibodies. Cells were cultured as described in Materials and Methods. Data from line 37 is taken from a separate experiment in which the control animal gave similar proliferative responses and inhibition but was not included for simplicity. The data is representative of four independent experiments. Numbers represent the mean of triplicate cultures after subtraction of background responses of the stimulator cells only and generally had a SD of 4–20%. Background responses in the absence of stimulator cells ranged from 140 to 350 cpm. Antibodies that do not react with stimulator cells exhibited up to 28% inhibition in different experiments. Thus, inhibition of ≤28% was considered to be nonspecific. Bold-faced numbers indicate significant inhibition. * inh, inhibition.

on A20.2J (data not shown). To determine whether the difference in stimulation was due to the species of cell on which the class II molecules were expressed, T cell responses to spleen cells from HLA-DQ3.2 transgenic mice and I-A^{bm12} mice were examined. The response to HLA-DQ3.2 was still lower than the response to I-A^{bm12} even when up to 25 times more DQ3.2 stimulator cells were used (Fig. 4 *A*). These results indicate that the murine T cell response to human class II molecules is a minimum of 25–70-fold lower than the response to allogeneic class II MHC molecules, based on the dose of stimulator cells required to give equivalent responses. Because culture conditions did not allow addition of sufficient cells to give a convincing xenogeneic response, the actual difference is almost certainly much greater than this value. The difference between allogeneic and xenogeneic responses is not due to the species of origin of the stimulator cells but instead reflects structural differences between murine and human class II molecules.

To determine whether the low responses observed in normal mice were due to a failure of murine CD4 to interact with human class II molecules, spleen cells from human CD4 transgenic mice were examined. In line 37 mice, which express a slightly lower than normal level of human CD4, T cell responses to the human class II molecules present on 721.221 (Fig. 3 *A*) and C1R (Fig. 3 *B*) were either slightly augmented or unaltered as compared to responses of littermate controls. However, in line 996 mice, which express a high level of human CD4, the responses to 721.221 and C1R were enhanced at least 10-fold, based on the doses of stimulator cells required to give equivalent responses (Fig. 3, *A* and *B*). Our ability to compare T cell responses to spleen cells expressing the human HLA-DQ3.2 molecule was limited by the high number

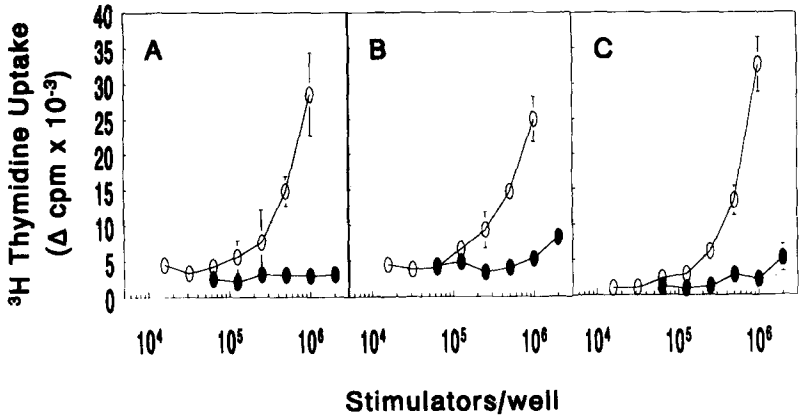

Figure 4. Responses to allogeneic and human class II transgenic spleen cells in normal and CD4 transgenic mice. Spleen cells from bm12 mice (*open circles*) and DQ3.2 transgenic mice (*closed circles*), both of which share the same background (H-2^b) were used as stimulators. Responder spleen cells were from (*A*) normal littermate control; (*B*) line 37; and (*C*) line 996. Cells were cultured as described in Fig. 3.

of stimulator cells required to induce significant proliferation and the relatively low xenogeneic responses obtained in all cases. Indeed, no observable response was ever detected in the littermate controls (Fig. 4). However, significant and increasing responses were observed at the highest stimulator doses used, in line 37 and line 996 (Fig. 4). In line 37 mice, responses to the allogeneic class II molecules expressed on A20.2J (Fig. 3 *C*) and I-A^{bm12} spleen cells (Fig. 4, compare *A* and *B*) remained unaltered as compared to responses of normal littermate controls. In different experiments using line 996, allogeneic responses were either unaffected (compare Fig. 4, *A* and *C*, and data not shown) or enhanced 1.5-fold to threefold (Fig. 3 *C*, and data not shown). The relatively greater enhancement of the response to human class II molecules suggests that human CD4 preferentially interacts with human class II molecules.

Despite the augmentation observed in line 996, the highest response to the human B cell lines 721.221 and C1R was still significantly less than the response to A20.2J. Allogeneic responses comparable in magnitude to the highest observed xenogeneic responses in line 996 were achieved using 16-33-fold fewer stimulator cells (compare Fig. 3, *A* and *B*, with *C*). In line 37, the level of the xenogeneic response was still so low that an accurate comparison could not be made. Similarly, maximal observed responses to DQ3.2 expressing murine spleen cells were comparable or less than those achieved using 20-fold fewer I-A^{bm12} expressing cells. Therefore, this difference was not due to the species of origin of the stimulator cell. Expression of human CD4 did not alter the specificity of these allogeneic and xenogeneic responses for class II molecules (Table 1). Thus, in every combination tested, responses to human class II molecules remained substantially lower than allogeneic responses in the same mice.

Participation of Human and Murine CD4 in Responses to Class II Molecules. The relative contribution of human and murine CD4 to T cell responses was examined by antibody blocking. As expected in normal mice, the response to class II alloantigens expressed on A20.2J was blocked with anti-murine CD4, but not by antibody against murine CD8 or human CD4 (Table 2). The response to the human class II molecules expressed on 721.221 was also inhibited by anti-murine CD4 to a comparable extent. In line 37 mice, responses to human class II molecules expressed in 721.221 were partially blocked by both anti-murine and anti-human CD4 (55% and 58%, respectively). In contrast, allogeneic responses to A20.2J (Table 2) and I-A^{bm12} (data not shown) were blocked by antibodies against murine but not human CD4. Surprisingly, anti-murine CD8 also reproducibly inhibited the response to A20.2J in line 37. Anti-murine CD8 did not inhibit the xenogeneic response in these mice. The response to A20.2J was still directed at class II and not class I molecules (Table 1). Possible reasons for this observation are discussed below. Nonetheless, the difference in patterns of inhibition of allogeneic and xenogeneic responses in line 37 are inconsistent with the notion that blockade is due to "negative signal transduction" and instead suggest that they reflect interference with ligand binding.

Interestingly, T cell responses of line 996 to either human or murine class II molecules were blocked only by anti-human CD4 antibodies, whereas neither anti-murine CD4 nor anti-murine CD8 had any effect. These results indicate that high level expression of human CD4 in this line impairs its ability to support normal responses to murine class II alloantigens, as well as human class II molecules. However, the level of allogeneic responses observed in these mice suggests that high-level expression of human CD4 is able to replace the

Table 2. *Antibody Sensitivity of the T Cell Response to Class II Antigens in Human CD4 Transgenic Mice*

			Responder [^{3}H]thymidine uptake (Δ cpm)					
Stimulator	mAb	Specificity	B6	% inh*	Line 37	% inh	line 996	% inh
A20 (murine)	None		34,942		102,675		60,380	
	GK1.5	Murine CD4	10,148	**71**	55,597	**46**	74,126	0
	Leu3a	Human CD4	32,785	6	137,393	0	5,643	**91**
	2.43	Murine CD8	29,040	17	40,785	**60**	49,215	18
721.221 (human)	None		5,087		8,951		27,305	
	GK1.5	Murine CD4	1,631	**68**	4,041	**55**	27,440	0
	Leu3a	human CD4	6,052	0	3,773	**58**	3,083	**89**
	2.43	Murine CD8	5,276	0	8,152	9	28,803	0

5×10^5 responder spleen cells were stimulated with 6.25×10^3 irradiated (5,200 rad) A20.2J cells or with 2.5×10^4 irradiated (9,000 rad) 721.221 cells either in the presence or absence of 50 μl of culture supernatant containing the indicated antibodies in a total volume of 200 μl. Cells were cultured as described in Materials and Methods. Data from line 37 is taken from a separate experiment in which the control animal gave similar proliferative responses and inhibition but was not included for simplicity. Numbers represent the mean of triplicate cultures after subtraction of responses of stimulator cells only and generally had a SD of 4–20%. Background responses in the absence of stimulator cells ranged from 140 to 350 cpm. Bold-faced numbers indicate specific inhibition.

* inh, inhibition.

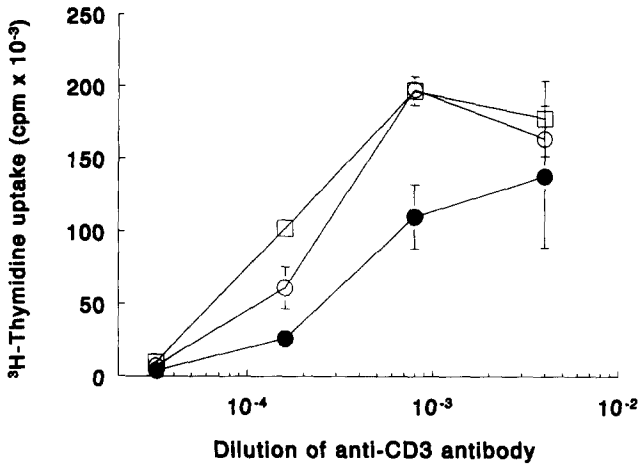

Figure 5. Synergism between murine CD4 and CD3 is diminished by overexpression of human CD4. Purified T cells from normal littermate control (*open squares*), line 37 (*open circles*), and line 996 (*closed circles*) were cultured in plates coated with sheep anti-mouse IgG and rabbit anti-rat IgG together with various dilutions of anti-CD3 and a 1:100 dilution of anti-murine CD4 supernatant. Background response to rat IgG control were subtracted from the responses against murine CD4. At the highest concentrations of anti-CD3 antibody, these responses were 40,000 cpm and ranged from 450 to 4,000 cpm at the three lowest dilutions. Bars, SD.

normal function of murine CD4. To determine whether the impairment was due to competition for factors involved in signal transduction, the ability of anti-murine CD4 to synergize with suboptimal concentrations of anti-CD3 was examined. Proliferative responses in line 37 were similar to those of normal mice (Fig. 5). This indicates that the signal transduction function of murine CD4 in line 37 is comparable to that of normal spleen cells. However, T cells from line 996 required 2–10 times more anti-CD3 antibody than T cells from line 37 to synergize with anti-murine CD4 antibody in stimulating comparable levels of T cell proliferation. Nonetheless, maximal proliferative responses in line 996 were similar to those of line 37 and normal mice. This indicates that the ability of murine CD4 to participate in signal transduction events has been somewhat reduced but not abolished.

Discussion

In this paper, human CD4 transgenic mice were utilized to determine whether low murine T cell responses to human class II molecules were due to a failure of murine CD4 to bind to these molecules. This approach differs from those previously employed to address this issue. Two different lines of transgenic mice were utilized, one of which expressed a slightly lower than normal amount of human CD4, whereas the other expressed a relatively high level. In both lines, the human CD4 molecule was able to synergize with murine CD3 in T cell activation, and the level of activation achieved was correlated with the amount of human CD4 expressed. In addition, in the transgenic line expressing high levels of human CD4, antibody against murine CD4 no longer blocked antigen recognition and its ability to synergize with murine CD3 was somewhat reduced. The exact mechanisms involved in the synergistic response have not been determined. However, it seems most likely to result from an interaction between CD4 and p56^{lck}, as well as additional interactions with components of the TCR complex. Our data would thus suggest that human CD4 can interact with both p56^{lck} and the TCR in transgenic T cells, and can effectively compete with murine CD4 for p56^{lck} binding and TCR access in order to enable its participation in T cell responses. Additional support for this conclusion comes from previous studies that have demonstrated that murine p56^{lck} associates with human CD4 in a transient coexpression system (31).

Although suggesting that human CD4 does interact with these components, our data do not directly indicate whether the level of interaction is comparable to that of murine CD4. Indeed, a comparison of the data in Figs. 2 and 5 might suggest that there is a greater degree of synergism between CD3 and murine CD4 than between CD3 and human CD4. However, this comparison is not valid because of necessary differences in the protocols used to attach the antibodies to the plates. In particular, anti-human CD4 and anti-CD3 are both attached to the plates through binding to the same sheep anti-mouse secondary antibody on the culture plates, whereas anti-murine CD4 is attached via an anti-rat reagent. Our conditions were optimized to give adequate synergism despite competition between anti-CD3 and anti-human CD4 for available binding sites. In addition, we have noted that not all antibodies against CD4 and CD8 are equivalent in their ability to synergize with anti-CD3 (our unpublished data). Nonetheless, we feel that the ability of human CD4 to effectively compete with murine CD4, combined with its ability to support allogeneic responses (discussed below), indicate a significant level of function for this molecule in transgenic mice.

The participation of human CD4 in murine T cell responses is also indicated by antibody blocking experiments. In the line expressing relatively low levels of human CD4, antibodies against this molecule blocked recognition of human, but not murine class II molecules. In the line expressing high levels, anti-human CD4 antibodies blocked responses to the class II molecules of both species, whereas antibodies against murine CD4 were without effect. Because murine CD4 continued to synergize with CD3 under these conditions, this result is not due to an inability to transduce signals leading to T cell activation. In addition, the specificity of antibody blocking observed in these experiments is inconsistent with an inhibitory mechanism based on negative signal transduction. Instead, inhibition appears to represent direct interference with the interaction between CD4 and class II molecules, and thus reflects the degree to which murine and human CD4 participate in antigen recognition. One incongruous observation was the unexpected but reproducible ability of anti-murine CD8 to inhibit the response to allogeneic class II molecules in line 37 mice. However, this same antibody failed to block the response to human class II molecules in the same mice, and had no effect on class II specific responses in normal and line 996 transgenic mice. Again, this specificity of the inhibition is inconsistent with a negative signal transduction mechanism. Although the exact mechanism is not

certain, it may reflect the participation of the murine $CD8^+$ subset, which in these mice also expresses human CD4. Efforts to examine the specificity and responsiveness of this subset are in progress.

Additional evidence of the ability of human CD4 to function as an effective coreceptor was provided by the observation that it augments antigen recognition in both transgenic lines. Responses to human class II molecules expressed on two different human B cell lines were enhanced up to threefold in the line expressing a relatively low level of human CD4, and a minimum of 10-fold in the high-level expresser. Responses to HLA-DQ3.2–expressing transgenic spleen cells were somewhat enhanced in both lines but to a lesser extent. This probably reflects the lower level of class II expression on spleen cells compared to B lymphoblastoid lines, as well as technical limitations on the number of spleen cells that could be added to cultures. Nonetheless, the allogeneic response in the same animals was either unaffected or augmented to a significantly smaller degree. It should be noted that the level of murine CD4 expression is reduced by 50% in both lines, but that the total CD4 density is higher than on normal animals. These results indicate that the level of allogeneic and xenogeneic responses cannot be simply correlated with the overall density of CD4 molecules regardless of their species of origin. Instead, the expression of human CD4 selectively augments responses to human class II molecules and argues for a preferential interaction between them.

Despite the fact that murine CD4 expression is reduced by 50% in both transgenic lines, there is no reduction in the allogeneic response. This suggests that either the reduced amount of murine CD4 is still sufficient to allow a normal response or that human CD4 is able to support the allogeneic response. An interaction between human CD4 and murine class II molecules is suggested by the normal to weakly augmented allogeneic response observed in line 996. Antibody blocking experiments indicate that this response is entirely dependent upon human and not murine CD4. It is important to recognize that the level of human CD4 expression in line 996 is significantly higher than normal. Therefore, these results do not in themselves allow an assessment of how well human CD4 binds to murine class II molecules. However, in combination with the data on augmentation of xenogeneic responses, we suggest that human CD4 binds to class II molecules of both species, but preferentially to those of human origin.

Three previous studies have addressed the issue of human CD4 function and antigen recognition in murine T cell hybridomas. In one case, it was shown that human CD4 binding to human class II molecules on an antigen presenting cell could augment recognition of an unrelated antigen (32). This presumably occurs through an augmentation of intercellular adhesion. In a second study, the ability of human CD4 to augment recognition of a human class II molecule by a murine T cell hybridoma was shown (33). However, in neither case was the recognition of murine class II molecules assessed. In a third study, it was found that transfection of human CD4 into a murine T cell hybridoma was as effective as transfection of murine CD4 in restoring recognition of murine class II molecules (34). Our data are not inconsistent with this conclusion, particularly if the level of human CD4 expression is high.

The data presented here also establish that antibody against murine CD4 blocks the response of normal C57BL/6 mice and of line 37 transgenics to both murine and human class II molecules. This suggests that murine CD4 does interact at some level with human class II molecules. This result appears to conflict with a previous report that murine CD4 does not functionally interact with human class II molecules (35). Furthermore, it appears inconsistent with the hypothesis on which the present study was based, i.e., that murine CD4 does not bind to human class II molecules. However, in the study cited (35), CD4 function did not require its interaction with the TCR, but only the ability to augment intercellular adhesion. It is also important to recognize that as with human CD4, the species specificity of murine CD4 may be preferential rather than absolute. The response to human class II molecules that is blocked by anti–murine CD4 is very weak, and this could reflect a limited participation by the murine CD4 molecule. The ability of human CD4 to specifically augment responses to human class II molecules provides further evidence that a poor interaction with murine CD4 molecules plays a role in limiting the xenogeneic response.

Despite the fact that expression of human CD4 augments murine T cell responses to human class II molecules, these never reached the level of the responses to allogeneic class II molecules. The augmentation observed in line 37 was surprisingly modest, because the level of human CD4 expression in these mice approximated that found on normal human PBL. This could indicate that although human CD4 functions in the murine T cell environment, it is relatively inefficient in comparison with murine CD4. However, in line 996, the response to human class II molecules was still 16–33-fold lower than the allogeneic response. This was so despite the fact that the high-level expression of human CD4 had uncoupled murine CD4 from the process of antigen recognition, and human CD4 fully supported the allogeneic response. These results indicate that the poor xenogeneic response cannot be overcome simply by optimizing species-specific interactions between class II molecules and the CD4 molecules expressed on peripheral T cells. This conclusion is in accord with earlier work from this laboratory in which xenogeneic responses to human class I molecules were examined utilizing human/mouse chimeric class I molecules (13), but contrasts with similar studies in which it was concluded that weak responses to xenogeneic MHC class I molecules were due to failure of CD8 to interact with HLA class I molecules and not to the selection of the mouse T cell repertoire (20). Instead, we conclude that the weak xenogeneic response to both class I and class II molecules must reflect intrinsic differences in murine and human T cell receptor structure, or the need for dual expression of human coreceptors and MHC molecules for optimal selection of human MHC-specific T cells during thymocyte development.

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